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Attorney Docket No. 1332

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:

David Bernard Fischer Joseph Kevin Gogerty [≪]Date:

February 28, 2003

Serial No.:

09/758;859

Group Art Unit:

1638

Filed:

¹January 11, 2001

Examiner:

-David T. Fox

For:

"INBRED MAIZE LINE PH7CP"

Assistant Commissioner for Patents Washington, D.C. 20231

- RULE 132 DECLARATION
OF
DR. DINAKAR BHATTRAMAKKI

Sir:

- I, Dinakar Bhattramakki, Ph.D., do hereby declare and say as follows:
- 1. I am skilled in the art of the field of the invention. I have a Ph.D. in Plant Molecular Genetics from the University of Illinois at Urbana-Champaign. I have a Bachelor of Science degree in Agricultural Sciences from the University of Agricultural Sciences, Bangalore, India. Since 1997 I have been engaged in the analysis of molecular markers for plants. I have supervised the Molecular Marker Applications lab at Pioneer Hi-Bred International, Inc. from January 2002 until the present.
- 2. I am familiar with the methods used in the analysis of Simple Sequence Repeat, SSR marker data for inbred PH7CP conducted at Pioneer Hi-Bred International, Inc. The analysis of the SSR profile of inbred PH7CP may be accomplished without any undue experimentation. The SSR profile for inbred PH7CP is attached hereto.
- 3. Means of performing this genetic marker profile are well known in the art. SSRs are genetic markers based on polymorphisms in nucleotide sequences. The PCRTM detection of SSRs is accomplished by using two oligonucleotide primers flanking the polymorphic segment of DNA. Amplification is accomplished through repeated cycles of heat denaturation of the DNA followed by annealing of the primers to their complementary sequences at low temperatures, and extension of the annealed primers with DNA polymerase.

- 4. Markers are scored following amplification and gel electrophoresis of the amplification products. Scoring of marker genotype is based on the size or weight of the amplified fragment. While variation in the primer used or in laboratory procedures can affect the reported marker score, relative values remain constant regardless of the specific primer or laboratory used.
- ovailable and may be used to identify the SSR markers reported herein are publicly available and may be found in the Maize DB on the World Wide Web at agron.missouri.edu/maps.html (sponsored by the University of Missouri), in Sharepova et al. (Plant Mol. Biol. 48(5-6):463-481) and/or in Lee et al (Plant Mol. Biol. 48(5-6); 453-461).

 Markers shown for PH7CP are the publicly available markers in the sources listed above for which PH7CP was tested and shown to be homozygous.
- 6. Map information is provided by bin number as reported in the Maize DB. The bin number digits to the left of decimal point typically represent the chromosome on which such marker is located, and the digits to the right of the decimal typically-represent the location on such chromosome.
- Thereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: February 28, 2003

-Dinakar Bhattramakki

| | | | 1705 |
|------------------|----------------|---------|----------------------|
| Public Name of | · • | | 17CP |
| Marker | Di | | se pairs 129.38 🖘 |
| phi427913 | | 1.01 | 129.30 |
| bnlg1014 > | | 1.01 | |
| phi056 👉 😞 | - Ju | 1.01 | 255.30 |
| b <u>plg1083</u> | ~ | 1.02 | 223.73 |
| bnlg1127 | \$ | 1.02 | 96.21 🗢 |
| bnlg1953 🔔 💂 | | 1.02 | 205.80 |
| bnlg1429 | , 7 | 1.02 | 190.09 |
| bnig439 _ | | 1.03 | 232:00 |
| phi109275 | | 1.03 | 131.94 |
| phi339017_ | | 1.03 | 145.56 |
| bnlg1203 | | 1.03 | 306.51 |
| -bnjig1484 | | 1.03 | 143.49 |
| bnlg2086 | 1.4 | 1.04 | 224.54 |
| bnlg1832 | | 1.05 | 233.45 |
| bnlg1886 | | 1.05 | _ 141.48 |
| bnlg1057 | * | 1.06 | 270.49 |
| bnlg1041 | | 1.06 | 240:52 |
| bnlg1615 | | 1.06 | 232.40 |
| bnlg1556 | | 1.07 | 207.66 |
| phi323065 | | 1.08 | 329.53 |
| phi335539 | | 1.08 | - 88.58 |
| phi423298 | • | 1.08 | 133.68 |
| phi002 | | 1.08 | 69.78 |
| _bnlg1331 | | 1.09 | 121.03 |
| phi011 | | 1.09 | -226.98 |
| phi308707 | | 1.10 | 131.11 |
| phi227562 | | 1.11 | 322.45 |
| phi265454 | | ·. 1.11 | 217.66 |
| phi064° | | 1.11 | 80.39 |
| phi402893 | | 2.00 | |
| phi96100 | | 2.01 | -280.53 |
| bnlg1017 | | 2,02 | 1.95.57 |
| bnlg2277 | | 2.02 | _ 294.16 |
| bnlg1064 📜 🔔 | < ?" | 2.03 | 188.18 |
| bnlg1018 | - | 2.04 | 138.37 |
| _ bnlg1909 | | 2.05 | |
| bnlg1138 | - | 2.06 | ³ 223.89 |
| bnlg1396 | ۲, | 2.06 | • |
| bnlg1831 | | 2.06 | 185.86 |
| phi251315 | | 2.07 | 123.79 |
| phi328189 | | 2.08 | 121.35 |
| phi427434 | | 2.08 | 130.91 |
| phi435417 | | 2.08 | 214.27 |
| bnlg1141 | | 2.08 | 152.92 |
| bnlg1940 | | 2.08 | 247.33 |



| bnlg1520 | 2.09 | 296.30 |
|---------------|-------------------|----------|
| phi101049 | 2.10 | 226.43 |
| phi453121 | 3.00 | 217.80 |
| phi104127 | 3.01 | 169.96 🔭 |
| phi404206 | 3.01 | 300.24 |
| phi193225 | 3.02 | 136.71 |
| phi374118 _ | 3.02 | 222.89 |
| bnlg1144 - | 3.02 | 134.68 |
| bnlg1647 | 3.02 | 131.00 |
| bnlg1523 | 3.03 | 264.89 |
| _bnlg1019 | 3.04 | - 178.28 |
| ⇒ bnlg1035 | 3,05 | 112.85 |
| phi053 | 3.05 | 191.64 |
| phi102228 | 3.06 | _ 135.04 |
| bnlg1160 | 3.06 | 222.23 |
| - | 3.06 | 126.99 |
| bnlg1951 . | 3.06 | 142.63 |
| bnlg2241 | 4.00 | 139.43 |
| phi072 | | 284.60 |
| phi213984 | 4.01 | |
| - bnlg1162 | 4.03 | 104.11 |
| - phi308090 | 4.04 | |
| phi438301 | 4.05 | 208.45 |
| bnlg1159 | 4.05 | 147.81 |
| bnlg1755 | 4.05 | 214.54 |
| _bnlg1937 | 4.05 | 237.92 |
| _ bnlg1265 | 4.05 | 233.26 |
| _ phi079 | 4.05 | 185.40 |
| bńlg1189 | 4.07 | 140.17- |
| bnlg2244 | 4.08 | 216.18 |
| bnlg1006 | 5.00 | 227.74 |
| phi396160 | 5.02 | 301.03 |
| phi109188 _ | 5.03 | 161.52 |
| bnlg653 | 5.04 | 153.96 |
| - phi330507 | 5.04 | 131.59 |
| * phi331888 | 5.04 | 130.72 |
| -bnlg1208 | ⁻ 5.04 | 118.88 |
| bnlg1892 ~ | 5.04 | 158.09 |
| phi333597 _= | ~ 5.05 | 210.74 |
| phi085 * | 5.06 | 248.06 |
| - bnlg1118 | 5.07 | 80.70 |
| 5nla1711 | 5.07 | 180.71 |
| phi423796 | 6.01 | 128.39 |
| phi389203 | 6.03 | 306.15 |
| phi452693 | 6.04 | 130.98 |
| phi445613 | 6.05 | 100.12 |
| bnlg1174 | 6.05 | 218.72 |
| phi364545 | 6.07 | 135.59 |
| bnlg1740 | 6.07 | 236.30 |
| 5/11g (1 1 0 | 4.4. | |

6.07 136.00 bnlg1759 78.29 phi070; umc1063 6.07 7.02 137.50 phi034 7.03 233.69 bnig2271 7.04 121.92 phi328175 **-7**.05 318.75 phi260485 7.05 138.57 phi051= 7.05 201.72 phi069 ... 7,06 168.82 phi116 8.00 291.88 phi420701 8.02 140.77 bnlg1194-5 8.03 172.52 bnlg2082 8.03 302.63 phi115 8.03 97,90 ~phi121 320.35 bnlg2046 8.04 217.94 8.05 bnlg1176 8.06 121.65 bnlg11,52 8.07 215.69 bnlg1065 8.08 110.49 bnlg1056 8.08 94.16 phi015 8.09 136.10 phi233376 bnlg2122 9.01 234.97 236.63 9.04 phi032 9.05 122.38 phi108411 122.93 9.05 phi236654 9.08 300.56 _ bnlg1129 202.94 phi041 10.00 252:35 10.02 phi96342 153.35 10.02 - phi059 10.03⁻ 170.34 bnlg1079 83.27 phi050 10.03 128.33 -10.04 phi301654 phi062 -10.04 161.07 phi323152 10.05 134.72 bnlg1074 161.94 10.05 151.61 bnlg1185 10.07 ⇒ bnlg1450 -10.07 189.35 137.61 phi109642 2.03/2.04 1.09-1.10 240.19

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Dec. 10, 2002

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Janūary 11, 2001

Examiner:

David TaiFox

For:

"INBRED MAIZE LINE PH7CF

Assistant Commissioner for Patents Washington, D.C. 20231

> RULE-132 DECLARATION - OF DR. STEPHEN SMITH

Sir:

- I, Stephen Smith, PhD., do hereby declare and say as follows:
- I am skilled in the art of the field of the invention. I have a Ph.D. in Biochemical 1. Systematics and Taxonomy of Maize and its Wild Relatives from Birmingham University I have a M.Sc. in the Conservation and Utilization of Plant Genetic Resources from Birmingham University. I have a Bachelor of Science degree in Plant Sciences from London University. Since 1977 I have been engaged in the development, study and application of molecular markers to genetics, measuring genetic diversity and tracking pedigrees. I commenced this work at North Carolina State University as a post-doctoral research fellow. I have continued my engagement in these studies during my employment by Pioneer Hi-Bred from 1980 until the present. These studies have resulted in numerous scientific articles that have appeared in peer reviewed scientific literature.
- I have read and understood the Office Action in the above case dated October 30, 2002. This declaration is in response to the Examiner's rejection under, 35 U.S.G.-S. 102(e) as anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as obvious over Bradbury (U.S. Patent No. 5,936,145).
- I have conducted an analysis of SSR marker data for inbred PH7CP and the inbred cited as prior art, 87DIA4. Out of a total of 76 SSR loci examined, which allowed a sampling of each chromosome, there are 44 markers that show differences between PH7CP and 87DIA4. This represents a difference for 58% for the markers tested. Of

Appendix F

these 44 markers, 23 were greater than 50 cM in distance, or unlinked on the genetic map.

- 4. Upon crossing PH7CP to any other maize line and selfing successive filial generations, one would within the realm of what is statistically possible, obtain a progeny inbred maize line that retains genetic-contribution from PH7CP. Assuming that (i) the cited prior art is used as the maize line to which PH7CP is crossed, (ii) that the only difference between PH7CP and 87DIA4 are these 44-markers, and (iii) that all markers within a 50 cM distance will segregate together, then the odds of obtaining a PH7CP progeny inbred that is the same as 87DIA4 after one cycle of breeding, is 1 in 2²³ or 1 in 8,388,608. Statistically it is extremely unlikely that a PH7CP progeny, after-one cycle of breeding, would be the same as 87DIA4.
- Further, the assumptions made above vastly overstate the likelihood of breeding PH7CP from 87DIA4. For example, it is common practice in quantitative genetics to determine the relation of plants by differences in markers. In doing so, one extrapolates that a percentage difference in markers is indicative of a difference in the whole genome. To assume that the only differences between PH7CP and 87DIA4 are for these 44 markers, when 44 markers constitute 58% of the 76 SSR loci examined, is a gross and unrealistic assumption. Further the current maize genetic map only has approximately, sixty 50cM units, so by applying this limitation the maximum number of independently segregating loci one could obtain, using the most different maize lines that could ever be found, is sixty. These assumptions result in an over estimate of the odds of breeding PH7CP from 87DIA4.
- 6. Given the difference in molecular markers between PH7CP and 87DIA4, it is my expert opinion that PH7CP and 87DIA4 are very distinct inventions. It is also my expert opinion that, within the realm of what is statistically possible, any progeny of PH7CP developed through crossing PH7CP with another plant will be distinct from 87DIA4. Given the facts and based on my education and scientific experience, I believe that the invention as claimed is not obvious nor anticipated by Bradbury (U.S. Patent No. 5,936,145).
- 7. Inhereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Orc 11 w 2002

Rv.

Stephen Smith

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